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Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency

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ABSTRACT

The influence of the proanthocyanidic, polysaccharide and oligosaccharide composition on astringency perception of Tempranillo wines has been evaluated.

Statistical analyses revealed the existence of relationships between chemical composition and perceived astringency. Proanthocyanidic subunit distribution had the strongest contribution to the multiple linear regression (MLR) model. Polysaccharide families showed clear opposition to astringency perception according to principal component analysis (PCA) results, being stronger for mannoproteins and rhamnogalacturonan-II (RG-II), but only Polysaccharides Rich in Arabinose and Galactose (PRAGs) were considered in the final fitted MLR model, which explained 96.8% of the variability observed in the data. Oligosaccharides did not show a clear opposition, revealing that structure and size of carbohydrates are important for astringency perception. Mannose and galactose residues in the oligosaccharide fraction are positively related to astringency perception, probably because its presence is consequence of the degradation of polysaccharides.

1. Introduction

Astringency is one of the most important attributes of red wine and it is close related to its overall quality, being widely acknowledged that high quality level red wines have a balanced level of astringency (Gawel, 1998). Astringency has been described as an oral sensation which causes the drying, roughing and puckering of the mouth epithelia and a complete terminology has been developed to describe this complex sensation in red wines (Gawel, Oberholster, & Francis, 2000). It has been classically attributed to the interaction between tannins and salivary proteins (Bate-Smith, 1954) leading to precipitation, although more recently other mechanisms, including the influence of colloidal particles that remain in solution and the involvement of laminin receptor (Schwarz & Hofmann, 2008) had been proposed.

Phenolic compounds present in wine, and especially tannins have been widely related to astringency perception (Kennedy, Ferrier, Harbertson, & Peyrot des Gachons, 2006; Brossaud, Cheynier, & Noble, 2001; Preys et al., 2006). Regarding proanthocyanidins, several variables, such as total concentration and the average polymerisation degree (aDP) (Preys et al., 2006), and their subunit composition and their distribution highly correlate with the astringency perception (Quijada-Morin et al., 2012).

Salivary proteins–tannin interactions appear to be the main astringency mechanism in red wines. Polysaccharides could inhibit this interaction in red wines, as it has been previously proposed for the loss of astringency in ripening fruits (Ozawa, Lilley, & Haslam, 1987). Those authors observed a release of small pectin soluble fragments as the cellular structure of fruit softens up during ripening, and they suggested that there is a competition with salivary proteins for polyphenolic substrates which lead to a modification in astringency perception and response. Several in vitro studies had been developed, showing that complex polysaccharides can disrupt protein–tannin interaction by different mechanisms; inhibiting protein–tannin interaction (Carvalho et al., 2006; Escot, Feuillat, Dulau, & Charpentier, 2001) or inhibiting the precipitation of the protein–tannin complexes (de Freitas, Carvalho, & Mateus, 2003; Mateus, Carvalho, Luis, & de Freitas, 2004), thereby polysaccharides would limit the concentration of available proanthocyanidins, and thus astringency would be reduced. In addition to this, several polysaccharide families had been described as compounds able to interact with tannins (Poncet-Legrand, Doco, Williams, & Vernhet, 2007; Riou, Vernhet, Doco, & Moutounet, 2002) or with proanthocyanidin aggregates to yield soluble complexes (Riou et al., 2002), so the presence of available proanthocyanidins in

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the medium would also be reduced by these mechanisms. These studies used different indirect approaches to show the influence of complex carbohydrates on protein–tannin interactions; such as nephelometry studies (Carvalho, Povoas, Mateus, & de Freitas, 2006; de Freitas et al., 2003; Mateus et al., 2004), light scattering (Carvalho et al., 2006), determination of gelatin index (Escot et al., 2001) or SDS–PAGE (Soares, Mateus, & de Freitas, 2012). Sensory analysis has been also used to study the influence of polysaccharides on astringency perception in model wine solutions (Vidal, Courcoux, et al., 2004; Vidal, Francis, et al., 2004), showing that all polysaccharide families reduced the perception of astringency in some degree (McRae & Kennedy, 2011). In addition to this, the above-mentioned sensory studies in model wine revealed that acidic polysaccharides have a greater impact on the reduction of astringency perception. RG-II is the main acidic polysaccharide in wines (Vernhet, Pellerin, Prieur, Osmianski, & Moutounet, 1996); isolated fractions of this polysaccharide caused a significant decrease of overall astringency in model solution, which has been attributed mainly to changes in mouth lubrication and the formation of complexes with astringent compounds. Neutral polysaccharides also tend to decrease the intensity of astringency attributes, nevertheless in that study the differences between model wine and the fraction containing a mixture of mannoproteins and type II arabinogalactan proteins isolated from wine, were not statistically significant (Vidal et al., 2004).

The aim of this work was to study the proanthocyanidic, polysaccharide and oligosaccharide composition of Tempranillo red wines and to establish relationships with perceived astringency.

2. Materials and methods

2.1. Wine samples

Thirteen commercial wines made from Tempranillo grapes were purchased from selected Spanish wineries. The wines belonged to four vintages (2006, 2008, 2009 and 2010), and three Spanish protected designations of origin, Ribera de Duero, Toro and Rioja (Table 1). All the samples are Tempranillo wines that have evolved during the ageing time. They were stored under cellar conditions before the analyses were conducted. Oenological parameters as pH or ethanol content were similar across the studied wines, and they were not included in the statistical data treatment.

2.2. Proanthocyanidin extraction

Bleaching of anthocyanins pigments was carried out as described by Alcalde-Eon, Escribano-Bailón, Santos-Buelga, and Rivas-Gonzalo (2004) with slight modifications. In brief, 2 mL wine were adjusted to pH 1.0 with a drop of concentrated hydrochloric acid, transferred to a 5 mL test tube containing 800 mg sodium bisulphite and stirred for 20 min. Under these conditions, most monomeric anthocyanins are combined with bisulphite to form colourless sulphonic acid adducts, which can be readily retained by anion exchange sorbents. Thus, the bleached wine was diluted 1:1 with ultrapure water and 2 mL were loaded into a mixed-mode anion exchange/reversed phase SPE cartridge Oasis MAX (60 mg, 3 mL) from Waters (Milford, MA, USA), previously conditioned with 2 mL 75% acetone in water followed by 4 mL water. After washing with 4 mL water, flavan-3-ols and proanthocyanidins were eluted with 8 mL 75% acetone in water, whereas anthocyanins and organic acids were still retained through anion exchange interactions. The eluate was brought to dryness on a rotary evaporator at 30 °C and then reconstituted in 200 μL methanol to obtain the methanolic wine extract.

In order to quantify flavan-3-ols in wine, 50 μL of this methanolic extract were filled up to 1 mL with 2.5% acetic acid in water, filtered by 0.20 μm and analysed by HPLC–DAD–ESI/MS.

2.3. Acid-catalysed degradation in presence of phloroglucinol

Proanthocyanidins extracted from wines were characterized following the acid-catalysed cleavage of the polymer in the presence of phloroglucinol excess according to the procedure described by Kennedy and Jones (2001) with minor modifications, as follows. A solution containing 0.2 M HCl, 50 mg mL^{-1} phloroglucinol and 10 mg mL^{-1} L-ascorbic acid was prepared in methanol as phloroglucinolysis reagent. 100 μL methanolic wine extract were allowed to react with 200 μL phloroglucinol solution in a water bath for 40 min at 50 °C. Afterwards, the reaction was cooled down and quenched by the addition of 2.7 mL of 15 mM sodium acetate aqueous solution.

The reaction mixture was then purified by SPE using an Oasis MAX cartridge (60 mg, 3 mL) previously conditioned with 2 mL 75% acetone in water followed by 4 mL water.

The cartridge was washed with 4 mL water and the phloroglucinolysis products were eluted with 8 mL 75% acetone in water. This eluate was evaporated to dryness on a rotary evaporator at 30 °C, reconstituted in 1 mL 2.5% acetic acid in water, filtered by 0.20 μm and analysed by HPLC–DAD–ESI/MS.

2.4. HPLC–DAD/ESI–MS analyses

Analyses were carried out in an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) consisting of an

Table 1
Grape cultivar, vintage, protected designation of origin and sensory-determined astringency of the selected red wines.

Wines	*Vitisvinifera* L. cv.	Vintage	PDO	Astringency[a]
T1	Tempranillo	2006	Toro	1.80 ± 0.45
R1	Tempranillo	2008	Rioja	1.86 ± 0.65
R2	96% Tempranillo, 4% Graciano	2008	Rioja	0.60 ± 0.32
R3	Tempranillo	2009	Rioja	3.43 ± 0.45
R4	Tempranillo	2010	Rioja	3.00 ± 0.58
D1	Tempranillo	2008	Ribera del Duero	2.60 ± 0.55
D2	Tempranillo	2008	Ribera del Duero	2.71 ± 0.81
D3	Tempranillo	2008	Ribera del Duero	2.00 ± 0.71
D4	Tempranillo	2008	Ribera del Duero	1.93 ± 0.59
D5	Tempranillo	2009	Ribera del Duero	3.43 ± 0.45
D6	Tempranillo	2010	Ribera del Duero	3.21 ± 0.49
D7	Tempranillo	2008	Ribera de Duero	4.40 ± 0.55
D8	Tempranillo	2008	Ribera de Duero	3.00 ± 0.71

PDO: protected designation of origin.
[a] Astringency scale from 0 (absence of astringency) to 5 (extreme astringency).

autosampler, a quaternary pump, a vacuum degasser, a thermostated column compartment and a diode array detector (DAD), and controlled by ChemStation software (version A.05.04; Agilent Technologies). UV/Vis spectra were recorded from 200 to 600 nm, while acquiring at a selected wavelength of 280 nm.

Chromatographic separation of the proanthocyanidin phloroglucinolysis products was performed on a reversed-phase column Spherisorb ODS-2 (150 × 4.6 mm, 3 μm) from Waters (Milford, MA, USA) maintained at 25 °C. Mobile phases A and B were respectively, 0.1% formic acid in water and acetonitrile. The following linear gradient was used to achieve the chromatographic separation: hold at 100%A for 2 min, decreased to 90%A over 23 min and hold for 2 min, decreased to 80%A over 20 min, decreased to 60%A over 10 min and hold for 3 min, decreased to 40%A over 5 min and decreased to 20%A over 5 min, then returned to initial conditions over 5 min and re-equilibrated for 3 min. The flow rate and the injection volume were set at 0.5 mL min^{-1} and 100 μL, respectively.

The HPLC system was coupled to a hybrid triple quadrupole/linear ion trap (QqLIT) mass spectrometer API 3200 QTrap (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V ionisation source and controlled by Analyst software (version 1.5; Applied Biosystems).

The mass spectrometer was operated in the negative electrospray ionisation (ESI) mode under the following specific conditions: ionspray voltage (IS), −3700 V; source temperature (TEM), 400 °C; curtain gas (CUR), 20 arbitrary units; ion source gas 1 (GS1), 40 arbitrary units; ion source gas 2 (GS2), 30 arbitrary units; declustering potential (DP), −25 V; entrance potential (EP), −10 V; cell exit potential (CXP), −3 V; and collision energy (CE), −20 eV. Nitrogen (>99.98%) was employed as curtain, ion source and collision gas. The detection was accomplished in the enhanced MS (EMS) full-scan mode, from *m/z* 100 to 1700, and in the enhanced product ion (EPI) mode, to obtain the corresponding full-scan MS/MS spectra.

Identification and quantification methodologies to ascertain flavan-3-ols (terminal subunits) and flavan-3-ols phloroglucinol adducts composition had been described in detail in our previous work (Quijada-Morin et al., 2012).

2.5. Isolation of polysaccharide and oligosaccharide fractions

The polysaccharide and oligosaccharide fractions were isolated as previously described (Ducasse, Williams, Meudec, Cheynier, & Doco, 2010). Tempranillo red wines (5 mL) were partially depigmented in polyamide CC 6 column, particle size 0.05–0.16 previously equilibrated with NaCl 1 M. Sugar compounds were eluted with NaCl 1 M while polyphenols were retained by the resin. Eluted fraction was concentrated under vacuum. Size exclusion high resolution column chromatography was performed by loading the concentrated fraction on a system composed by a 234-Gilson sampling injector (Roissy, France), an LC-10 AS Shimadzu pump (Kyoto, Japan) and a Isco Foxy sampling collector (Lincoln, NE, USA). Elution was performed on a Superdex-30 HR column (60 × 1.6 cm, Pharmacia, Sweden) with a precolum (0.6 × 4 cm) equilibrated at 1 mL min^{-1} with 30 mM ammonium formiate pH 5.6. Elution of polysaccharides and oligosaccharides was followed with an Erma-ERC 7512 refractive index detector. Polysaccharide fraction was eluted between 42 and 60 min, while oligosaccharide fraction was collected between 61 and 93 min. The isolated fractions were freeze-dried, redissolved in water and freeze dried again for three times to remove the ammonium salt.

2.6. Polysaccharide analysis

The polysaccharide composition was estimated from the concentration of individual glycosyl residues determined by GC–MS after hydrolysis, reduction and acetylation as described elsewhere (Apolinar-Valiente et al., 2013). Briefly, neutral monosaccharides were released after hydrolysis of the isolated polysaccharide fraction with 2 M trifluoroacetic acid (Albersheim, Nevins, English, & Karr, 1967) (75 min, 120 °C). They were then converted to their corresponding alditol acetates derivatives by reduction and acetylation and quantified using GC analysis using a fused silica DB-225 capillary column (30 m × 0.32 mm i.d., 0.25 μm film; J&W Scientific) with hydrogen as carrier gas, on a Shimadzu GC-2010 *plus* gas chromatograph. The alditol acetates were identified from their retention times by comparison with standard monosaccharides. Neutral sugars amounts were calculated relative to the internal standard (myo-inositol).

2.7. Oligosaccharides analysis

The isolated oligosaccharide fraction is undergone to solvolysis with anhydrous MeOH containing 0.5 M HCl for 16 h at 80 °C, followed by per-*O*-trimethylsilylation of the methyl glycoside derivatives in order to ascertain the neutral and acidic composition (Doco, O'Neill, & Pellerin, 2001). The TMS derivatives were separated on two DB-1 capillary columns (30 m × 0.25 mm i.d., 0.25 μm film) (temperature programming 120–200 °C at 1.5 °C/min), coupled to a single injector inlet through a two-holed ferrule, with H2 as the carrier gas on a Shimadzu GCMS-QP2010SE gas chromatograph. The outlet of one column was directly connected to a FID at 250 °C and the second column via a deactivated fused-silica column (0.25 m × 0.11 μm i.d.) was connected to a mass detector. Samples were injected in the pulsed split mode with a split ratio of 20:1. The transfer line to the mass was set at 280 °C. EI mass spectra were obtained from *m/z* 50 to 400 every 0.2 s in the total ion-monitoring mode using an ion source temperature of 200 °C, a filament emission current of 60 μA, and an ionisation voltage of 70 eV.

2.8. Sensory evaluation

The tasting panel was composed of seven wine professionals, including winemakers and enologists. Panelists were requested to evaluate the astringency of the studied wines on a scale from 0 to 5, with zero values being assigned when there was an absolute absence of astringency and an intensity score of 5 representing an extreme astringency. Alum was used as reference standard. Two of the previous training sessions were conducted in order to standardize criteria among the panel members. Astringency evaluation was carried out in two different sensory sessions with six and seven wines, respectively. Wines were presented at room temperature in wineglasses randomly coded with three-digit numbers. At the end of each session, all scorecards were collected and the average value was calculated (Table 1). In order to evaluate the consistency of the trained panel, one-way ANOVA was carried out with the scores given by them. The variance of the astringency scores is divided in two components, the one related to wine and the other related to panelist. Wine component causes 99.43% of the astringency variance, while panelist component causes 0.57% of variance, supporting the reliability of the sensory panel.

2.9. Statistical analysis

Unsupervised methods are applied to observe patterns in the data indicating relationships between samples and/or between variables (Brereton, 2003). The unsupervised pattern recognition method used for data analysis was principal components analysis (PCA), which was applied to the correlation matrix of the original variables.

Backward stepwise multiple linear regression was performed in order to develop a model for astringency perception using as independent variables wine compositions.

The SPSS 13.0 for Windows software package (SPSS, Inc., Chicago, IL) was used for data processing.

3. Results and discussion

3.1. Proanthocyanidin composition

Acid-catalysed depolymerisation in the presence of phloroglucinol was perform in order to obtain information about the proanthocyanidin subunit composition (Fig. 1a), average degree of polymerisation (aDP) (Fig. 1b), average molecular weight (aMW) and total proanthocyanidin concentration of these wines (Fig. 1c), as previously described (Quijada-Morin et al., 2012).

3.2. Polysaccharide composition

The concentration (mg/L) of Mannoproteins (MPs), Polysaccharides Rich in Arabinose and Galactose (PRAGs,) type II Rhamnogalacturonan (RG-II) and Glucans in wines was estimated from the concentration of individual glycosyl residues, determined by GC after hydrolysis, reduction and acetylation (Ducasse et al., 2010). Total polysaccharides contents (sum of individual polysaccharides families) ranged from 180 to 525 mg L^{-1} (Fig. 2a). In general, older samples showed a lower polysaccharide content, which may indicate that these compounds tend to decrease during wine storage (Doco, Quellec, Moutounet, & Pellerin, 1999). As stated previously, a decrease in total polysaccharide contents takes place after the end of malolactic fermentation (Guadalupe & Ayestaran, 2007). In general, Ribera del Duero samples had a lower content in comparison with Rioja samples. The only Toro protected origin sample presents an unusual high content, specially taking into account that it is the oldest sample in the study.

Glucans were the less abundant polysaccharide family in all the studied samples, their content ranged from 6.6 to 21.3 mg L^{-1}, involving 3.6% ± 1.0 of total quantified polysaccharides.

RG-II presented values ranging from 63 to 142 mg L^{-1}. The only Toro sample presented the highest value (141.7 mg L^{-1}). Values determined in Ribera del Duero samples were slightly lower than those in Rioja samples (63.2–115.1 mg L^{-1} versus 92.0–115.2 mg L^{-1}). Ageing seem to have a small influence on RG-II content since 2010 samples presented a slightly lower value than the rest of the samples with the exception of sample D5 which showed an unusual low value for this polysaccharide. These values are in good agreement whit those reported for other varieties as Merlot (Ducasse et al., 2010), but were lower than those determined in Monastrell (Apolinar-Valiente et al., 2013).

PRAGs values showed great differences between samples, ranging from 22.7 to 278.9 mg L^{-1}. In general, youngest wines presented higher percentages of this kind of compounds. Previously it has been stated that these compounds present a maximum value in wines 30–60 days after ending alcoholic fermentation and then their concentrations start to decrease (Guadalupe & Ayestaran, 2007). This behaviour could explain the differences between vintages observed in our samples. Nonetheless, we could observe too differences between production areas, Ribera del Duero wines presented lower levels than Rioja wines, and Toro wine presented a

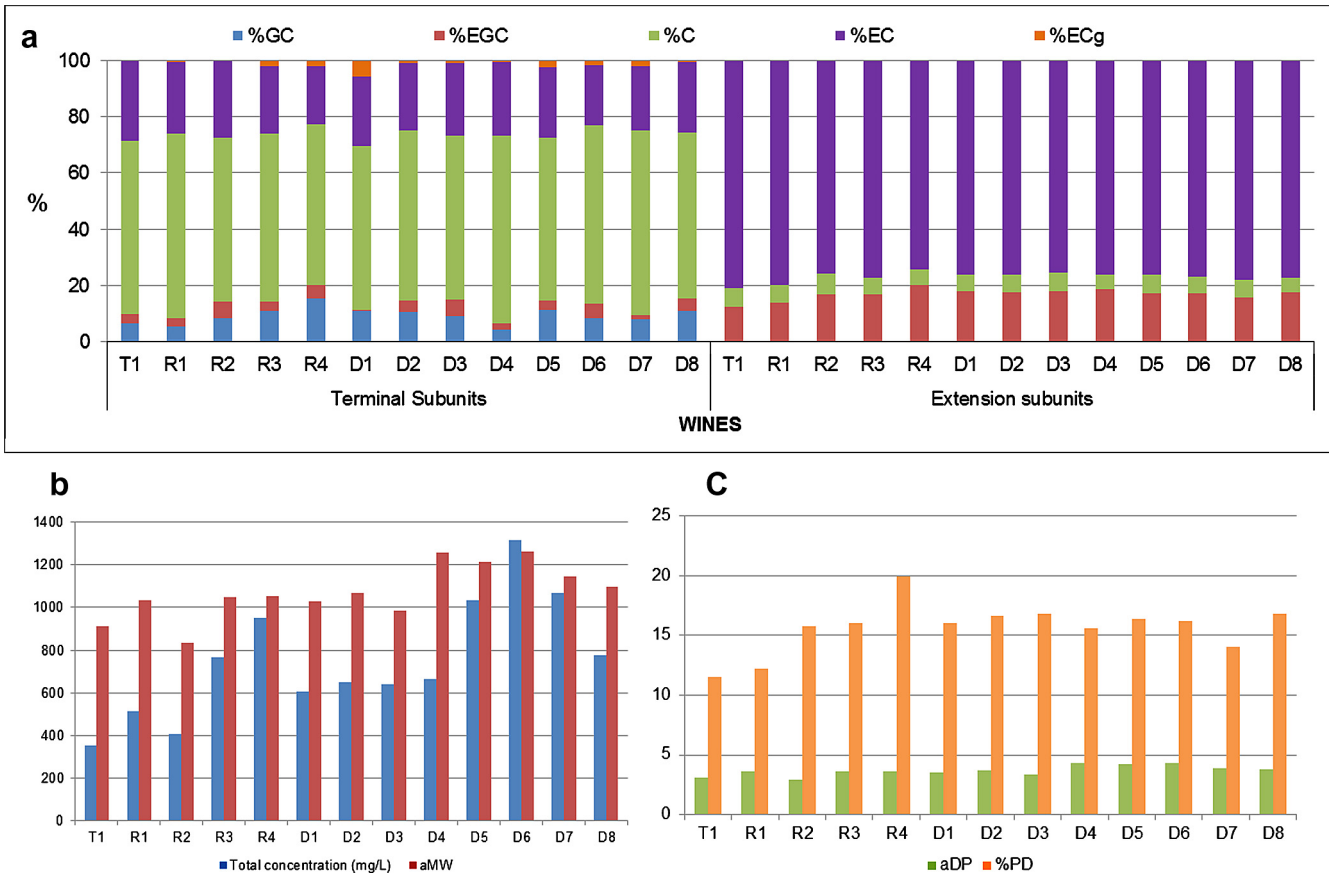

Fig. 1. Percentages (a) of proanthocyanidin subunits determined in Tempranillo red wines, total proanthocyanidin concentration, average molecular weight (aMW) (b), average degree of polymerisation and percentage of prodelphinidin subunits (c).

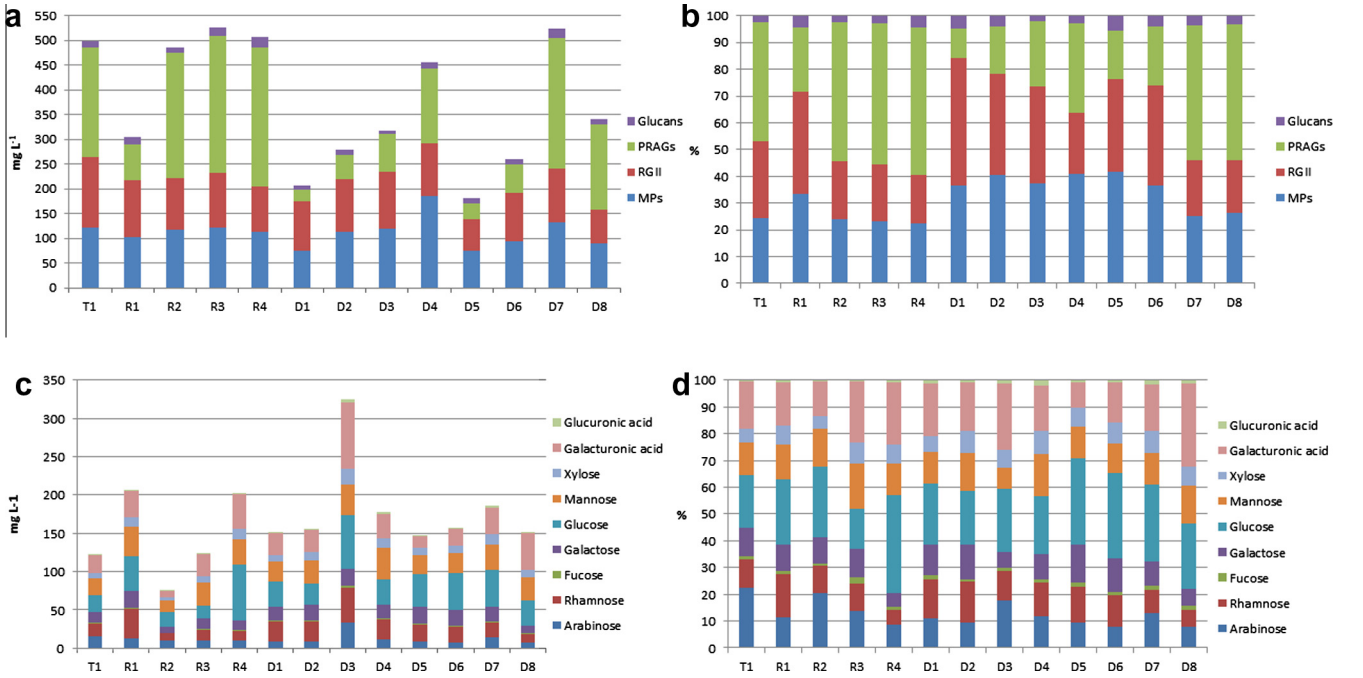

Fig. 2. Concentrations (a) and percentages (b) of the different polysaccharides families. Polysaccharides Rich in Arabinose and Galactose (PRAGs), Rhamnogalacturonan-II (RG-II), Mannoproteins (MPs). Concentrations (c) and percentages (d) of the different glycosyl residues determined in wine oligosaccharide fraction.

high level of this compounds, specially taking into account that it is the oldest wine in this study. This unusual high level may suggest that this wine presented a very high concentration of these compounds in its earlier ageing stages or the degradation processes in this wine during ageing were extremely low, or a combination of these two factors. The arabinose/galactose ratio, which is characteristic of PRAGs, was calculated, and differences in this ratio were observed across the studied wines. Younger wines (R3, R4, D7 and D8) and R2 wine presented the highest values for this ratio, ranging from 1.09 to 1.28, while older wines ratios were significantly lower ($p < 0.05$), varying from 0.35 to 0.67. Differences in arabinose/galactose ratio had been previously attributed to winemaking techniques (Doco, Williams, & Cheynier, 2007), but abovementioned results revealed that wine age has also influence in this ratio. Mannoprotein values ranged from 75.2 to 186.2 mg L^{-1}. These values are in good agreement with those previously determined by other authors in Tempranillo wines (Ayestaran, Guadalupe, & Leon, 2004), Merlot wines (Ducasse et al., 2010), Maturana and Monastrell wines (Guadalupe, Martínez-Pinilla, Garrido, Carrillo, & Ayestarán, 2012) and Monastrell variety (Apolinar-Valiente et al., 2013). No differences were observed according to the vintage or the production area of the wines in mannoprotein content, but Rioja wines values were closer between them than Ribera del Duero concentrations, which showed a greater dispersion. This kind of compounds is released from yeast cell walls during fermentation and ageing of wines, so the used yeast stain and vinification conditions such as the use of enzymes or heated treatments, seem to have an stronger influence on its levels (Apolinar-Valiente et al., 2013; Ayestaran et al., 2004; Doco et al., 2007), while grape variety or vintage have a minor influence. According to Guadalupe and Ayestaran (2007), the presence of mannoprotein in wines is increased from the end of the alcoholic fermentation to the end of malolactic fermentation and then remains nearly constant after 2 years of bottle ageing.

Percentages of the different families (Fig. 2b) in the studied wines revealed that PRAGs are the major compounds in the youngest wines (2009 and 2010 vintages), and in R2 and T1 wines. In general 2008 wines from Ribera del Duero presented high mannoprotein and RG-II percentages. T1 wine presents an unusual distribution, with high PRAG, medium RG-II and low mannoprotein percentages. All the studied wines presented low glucans percentages.

3.3. Oligosaccharide composition

Total oligosaccharides contents ranged from 75 to 325 mg L^{-1} (Fig. 2c), these results are slightly lower than those previously reported for other varieties like Merlot or Carignan (Ducasse et al., 2010), but they were similar or higher than those reported for Grignolino and Chardonnay (Bordiga et al., 2012). Nevertheless, glycosyl residues in the oligosaccharide fraction expressed in molar percentages are quite similar between them, with the highest differences for glucose, galacturonic acid and arabinose, and are also in good agreement with those reported for Merlot wines. To our knowledge, oligosaccharide profile of Tempranillo red wines is detailed here for the first time.

Nine different glycosyl residues were detected and quantified in all the analysed samples. Galacturonic acid, glucose and mannose presented the highest contents in all the studied wines; however, they showed great differences in contents between samples. Glucose was the major compound in seven samples, and its contents ranged from 16 to 73 mg L^{-1}. Mannose and galacturonic acid were the most abundant compound in three samples each one, and their contents reached values from 15 to 40 mg L^{-1} and from 9 to 86 mg L^{-1}, respectively. It is noteworthy that glucose and mannose, which are the most abundant compounds in 10 of the 13 samples, are mainly released from yeast cell walls while the rest of glycosyl residues in the oligosaccharide fraction are originated from grape cell wall polysaccharides. The contents of glucose and mannose in molar percentage varied from 31% to 48% (Fig. 2d).

Xylitol and 4-*O*-methylglucuronic acid that had been previously reported in low amounts in the oligosaccharide fraction of Merlot and Carignan wines (Ducasse et al., 2010) were not detected in the analysed Tempranillo red wines.

4-*O*-methylglucuronic acid is a characteristic sugar that accompanies galacturonic acid and xylose in glucuronoxylans. Its absence

may indicate that the oligosaccharides structures derivated from the hydrolysis of glucuronoxylans are not present in the studied samples.

Glucuronic acid and fucose were the less abundant glycosyl residues in the oligosaccharide fraction in all the studied samples.

3.4. Relationship between composition and perceived astringency

PCA analyses were used as unsupervised pattern recognition to reveal the possible relationships between the studied variables. Three loading plots were constructed (Fig. 3) relating perceived astringency and the three groups of compositional variables (i.e. proanthocyanidins, polysaccharide families and glycosyl residues in the oligosaccharide fraction).

Regarding the loading plot which relates proanthocyanidin composition and perceived astringency (Fig. 3a), ECg in terminal position (ECg-Term) contents is situated quite close to astringency, thus, this subunit was positively related with perceived astringency. ECg in terminal position was the only galloylated subunit found in our samples. In previous studies, the presence of galloylation has been strong and positively related to astringency perception (Preys et al., 2006). As can be seen in the loading plot, EGC in terminal position (EGC-Term) is related with a slight astringency perception. A negative relationship between EGC percentage and astringency had been also reported by Preys et al. (2006).

All polysaccharides families' contents showed opposition with perceived astringency (Fig. 3b) and are located in the loading plot opposed to astringency along PC1 which represents 54.5% of variability, being this effect stronger for RG-II and mannoproteins (MPs) since they are opposed along PC1 and PC2, which means 74.5% of the variability.

Mannoproteins are released from yeast cell walls and they have been described as compounds able to interact with polyphenols aggregates as steric stabilizers (Poncet-Legrand et al., 2007). It is possible that this feature is related to the ability of mannoproteins to smooth the astringency perception, since in this way proanthocyanidins would not be available to interact with salivary proteins, developing the astringency perception.

RG-II also showed strong opposition to astringency perception. This group of polysaccharides had been previously described as being able to decrease overall astringency of model wine (Vidal et al., 2004), nevertheless the negative relationship between RG-II and overall astringency is here described in wines for the first time. These polysaccharides present some unusual sugars and linkages between them, and they are the most branched complex polysaccharide present in wine (Pellerin et al., 1996). It is possible that

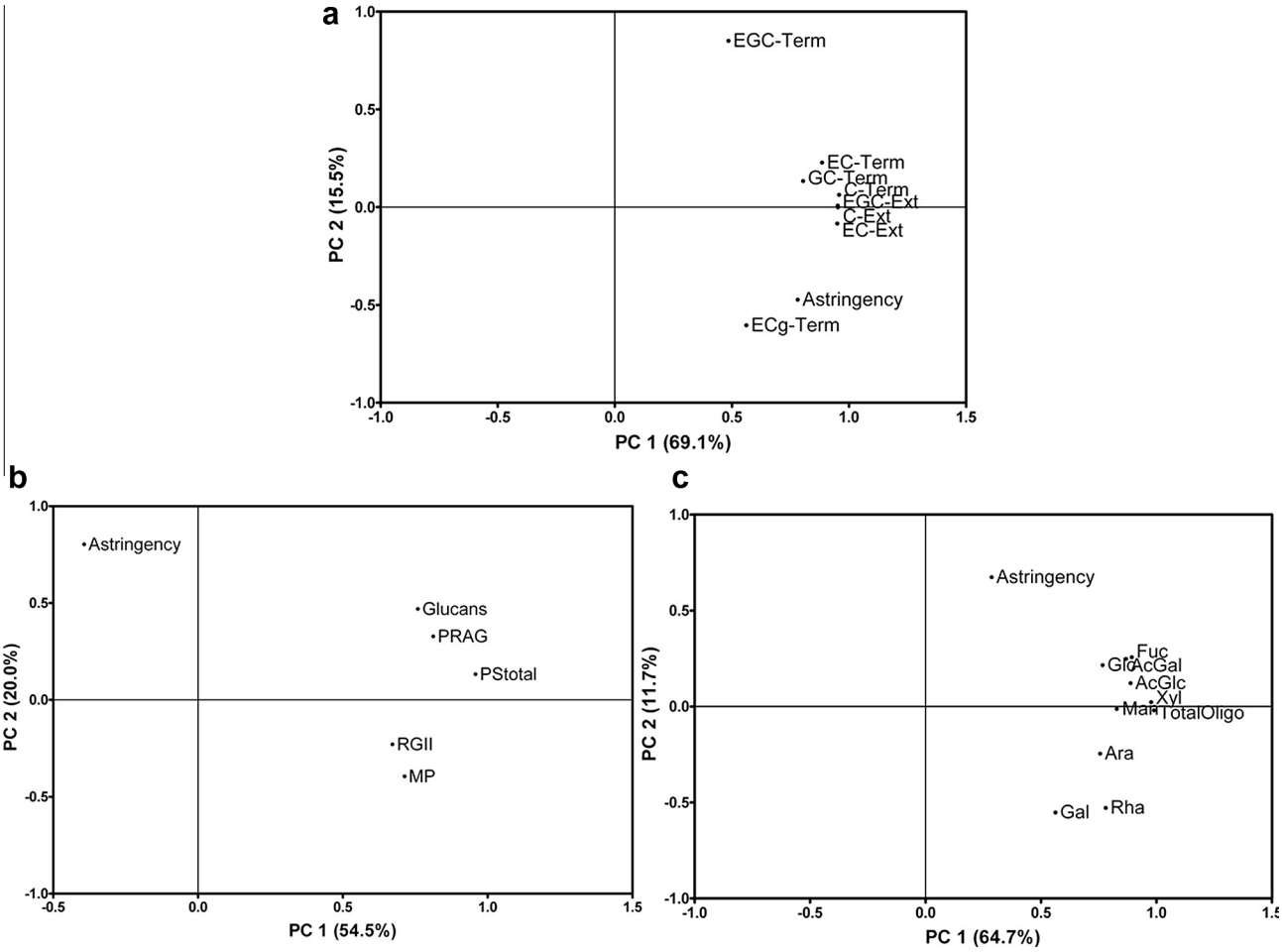

Fig. 3. Loading plots of the principal component analysis relating perceived astringency and proanthocyanidin subunit concentrations (a), polysaccharide families concentrations (b) and glycosyl residues in the oligosaccharide fraction concentrations (c). Catechin in extension positions (C-Ext); epicatechin in extension positions (EC-Ext); epigallocatechin in extension positions (EGC-Ext); catechin in terminal positions (C-Term); epicatechin in terminal positions (EC-Term); gallocatechin in terminal positions (GC-Term); epigallocatechin in terminal positions (EGC-Term); epicatechin-3-*O*-gallate in terminal positions (ECg-Term); Polysaccharides Rich in Arabinose and Galactose (PRAGs); Rhamnogalacturonan-II (RG-II); Mannoproteins (MPs); Total polysaccharide concentration (PS Total); Arabinose (Ara); Galacturonic acid (AcGal); Glucuronic acid (AcGlc); Fucose (Fuc); Galactose (Gal); Glucose (Glc); Mannose (Man); Rhamnose (Rha); Xylose (Xyl).

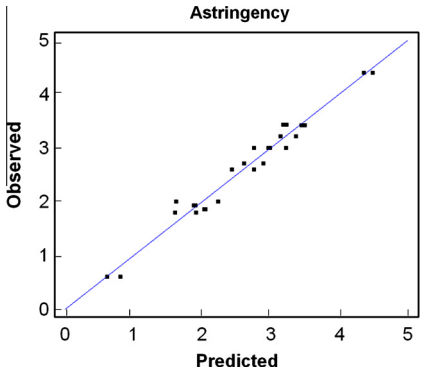

Fig. 4. Observed versus predicted plot for the astringency perception of the studied red wines.

both their branched structure and the presence of unusual sugars could be related to the smoothing of the astringency perception.

Glycosyl residues concentrations originated from the oligosaccharide fraction did not show a clear trend in the PCA plot constructed with astringency scores (Fig. 3c). It is noteworthy that all glycosyl residues found in oligosaccharide fraction are also found in the polysaccharide fraction, but their relation with perceived astringency is not clear. This fact may indicate that polymerisation and tridimensional structure are both important in the modulation of perceived astringency caused by polysaccharides, so oligosaccharides are not able to induce changes in astringency perception, while polysaccharides can modulate it.

Multiple linear regression was performed in order to evaluate the influence of compositional variables on perceived astringency and also to obtain a model with significant variables. Astringency was selected as dependent variable while compositional parameters such as proanthocyanidin subunit concentrations, polysaccharide families' concentrations and glycosyl residues originated from the oligosaccharide fraction concentrations were selected as independent variables. MLR was conducted applying a backward-stepwise strategy, which involves starting with all considered variables and removing the least significant one at each step of the process. The model is refitted after each step including only the most significant variables.

From the 21 initial independent variables, only nine were statistical significant in the final fitted model ($p < 0.05$). The regression coefficient R^2 took a value of 0.968, which supposes that the fitted model explains 96.8% of the variability observed in the data (Fig. 4). In a previous work 87.7% of variability of the perceived astringency was explained with the results of the proanthocyanidic composition (Quijada-Morin et al., 2012). The addition of the polysaccharide and oligosaccharide compositional variables implied an important improvement of the final model. The model provided also the β values (Table 2), which correspond to the standardized regression coefficients. Regarding these β values, proanthocyanidin subunit composition presented the highest β values, which implies that they are the most influent variables in the model. Epicatechin in extension positions presented the highest β value ($\beta = 1.388$). The positive sign of the β value indicated that there is a positive relationship between the amounts of epicatechin in extension positions and perceived astringency. Regarding the influence of prodelphinidin subunits, similar strength but opposite effects were observed, while GC in terminal position is positively correlated with astringency, EGC amounts in both terminal or extension positions would smooth perceived astringency. As it is has been mentioned above, the negative relationship between perceived astringency and EGC percentage in wines had been previously reported by Preys et al. (2006).

From all the polysaccharides families concentrations, only PRAGs showed statistical correlations with perceived astringency, despite RG-II and mannoproteins showed a higher opposition in the PCA analysis. Nevertheless, this is the variable with the smallest β-value included in the model, so its opposition to perceived astringency is quite small compared to the rest of factors, being 1.3 to 7 times smaller than other the factors. RG-II and mannoproteins were excluded from the model; this could happen because their relationship with perceived astringency does not follow a linear model so they are not good predictors in the fitted model. Glucans were considered as an independent variable in the initial model and were excluded in the first refitting step because of its low significance in the fitted model.

Four glycosyl residues corresponding to the oligosaccharide fraction showed statistical correlation with perceived astringency. Rhamnose (Rha) and xylose (Xyl) were negatively correlated with perceived astringency, while galactose (Gal) and mannose (Man) were positively correlated with it. High galactose concentrations in the glycosyl oligosaccharide composition seem to be related to a lower PRAG content. It is possible that the positive influence of galactose residues on perceived astringency is related with this decrease more than with the high galactose presence in the oligosaccharide fraction. A similar explanation could be thought for mannose and mannoproteins. An increase in mannose in the oligosaccharide fraction could be due to the degradation of mannoproteins, thus, these compounds would not be available to interact with proanthocyanidins. Those facts also support the idea of the importance of having the complete polysaccharide molecule for an effective interaction with proanthocyanidins, so the use of some enzymatic preparations in enology should be carefully studied to avoid undesirable polysaccharide degradations. Rhamnose and galactose presented β coefficients with similar absolute values (Table 2), which means that their influence in the fitted model has similar strength, but they presented different signs, so high rhamnose concentrations would reduce perceived astringency, while high galactose concentrations are related with a stronger astringency perception. Mannose and xylose presented similar and lower

Table 2
Results of the multiple linear regression analysis for the perceived astringency.

Parameter	Regression coefficient	β	Standard ERROR	*t*-Statistic	*p*-value
Constant	0.273764		0.458668	0.596867	0.5595
PRAGs	−0.00317074	−.195	0.00119188	−2.66029	0.0178
Rha	−0.0627012	−.459	0.019053	−3.29089	0.0050
Gal	0.0703083	.418	0.0123818	5.67837	0.0000
Man	0.0545639	.262	0.0172377	3.16538	0.0064
Xyl	−0.071691	−.293	0.0330659	−2.16813	0.0466
EGC-Ext	−0.0279056	−.711	0.00942086	−2.96211	0.0097
GC-Term	0.0696953	.648	0.0111288	6.26262	0.0000
EC-Ext	0.0105587	1.388	0.00161319	6.54522	0.0000
EGC-Term	−0.136334	−.648	0.0184121	−7.40458	0.0000

Abbreviations: PRAGs: polysaccharides rich in arabinose and galactose; Rha: rhamnose; Gal: galactose; Man: mannose; Xyl: xylose; EGC-Ext: epigallocatechin in extension positions, GC-Term: gallocatechin in terminal positions; EC-Ext: epicatechin in extension positions; EGC-Term: epigallocatechin in terminal positions.

β coefficients in absolute value than the other glycoside residues in the oligosaccharide fraction, so their influence to the model is smaller than the reported for rhamnose and galactose.

4. Conclusions

The results of this study confirm the influence of polysaccharides and oligosaccharides on perceived astringency in Tempranillo red wines. These compounds are able to modulate overall astringency.

The smoothing capacity of the different polysaccharides families is confirmed in wines for the first time. This effect is especially important for mannoproteins and RG-II. Glycosyl residues in the oligosaccharide fraction did not express a clear trend against astringency perception. Taking into account that all the glycosyl residues found in the oligosaccharide fraction are also found in the polysaccharide fraction, it seems that the ability of carbohydrates to smooth astringency perception is related to the size and tridimensional structure of the compounds.

Mannose and galactose concentrations in the oligosaccharide fraction are positively related to astringency perception; this could be related to a decrease in mannoproteins and PRAGs levels and not to a direct effect of these glycoside residues on astringency perception. The role that commercial enzymatic preparations could have over the degradation of polysaccharides leading to apparently lees protective oligosaccharides arises.

The regression model constructed involving the compositional variables and the perceived astringency allowed us to explain 96.8% of the variability observed in the data, and to recognize the variables that were positively and negatively related to astringency perception.

Further studies should be carried out to confirm the importance of size and tridimensional structure of carbohydrates in astringency perception.

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